

The use of oral fluids from pig populations for the diagnosis and monitoring of infectious disease



Optimising PRRSv RNA recovery from porcine oral fluids

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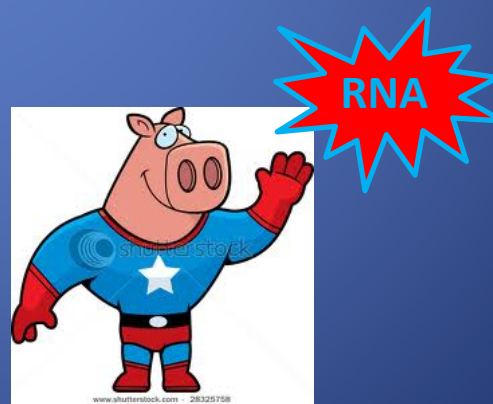


Introduction

Increasing development/commercialisation of swine OF diagnostics

Sample treatment post-collection can influence assay performance

RNA Degradation

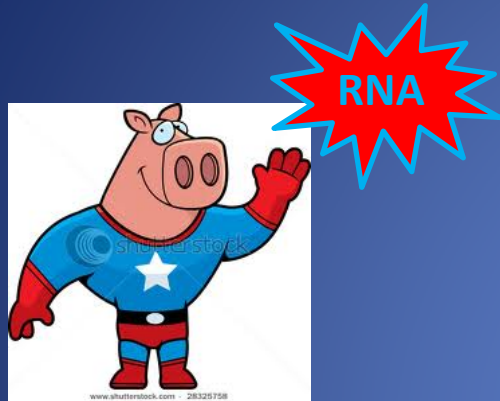


Objectives

- To develop a sample handling protocol for porcine OF while maintaining diagnostic viability
 - viral RNA for RT-PCR
 - ambient temperatures
 - simple to implement

3 Pilot Experiments

Protecting RNA



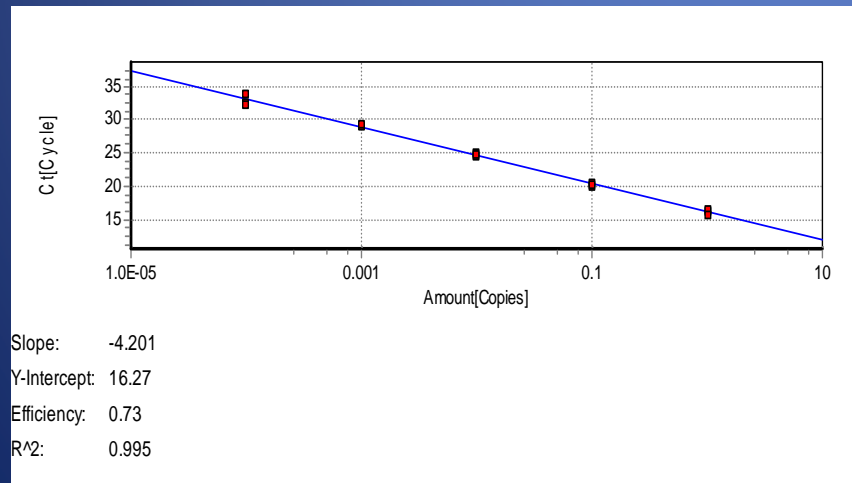
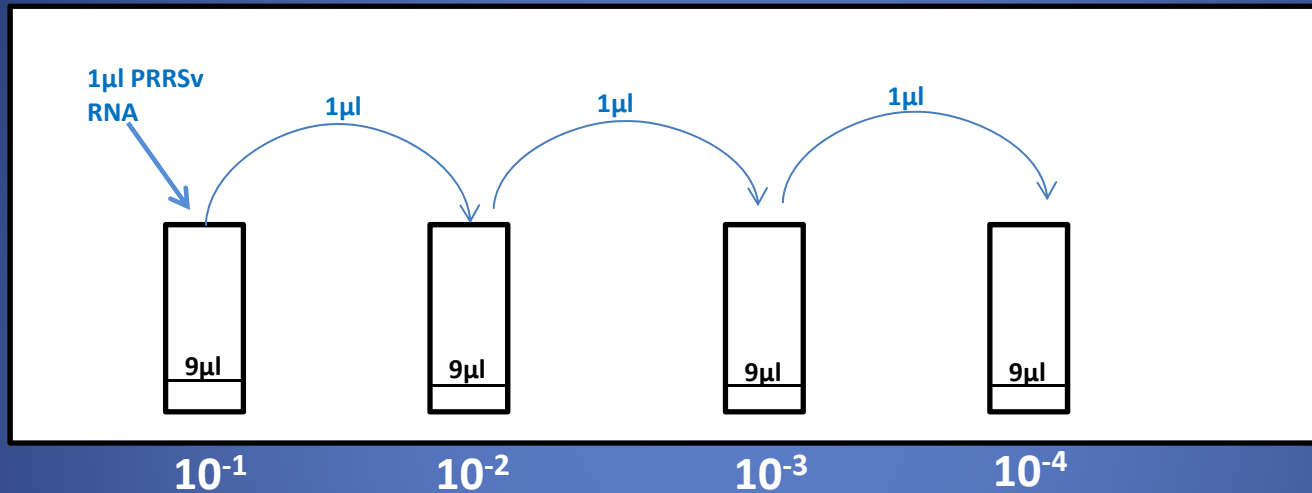
A treatment that should stop the degradation of RNA in saliva samples at the point of addition

In humans



Experiment 1: PRRSv RNA water test

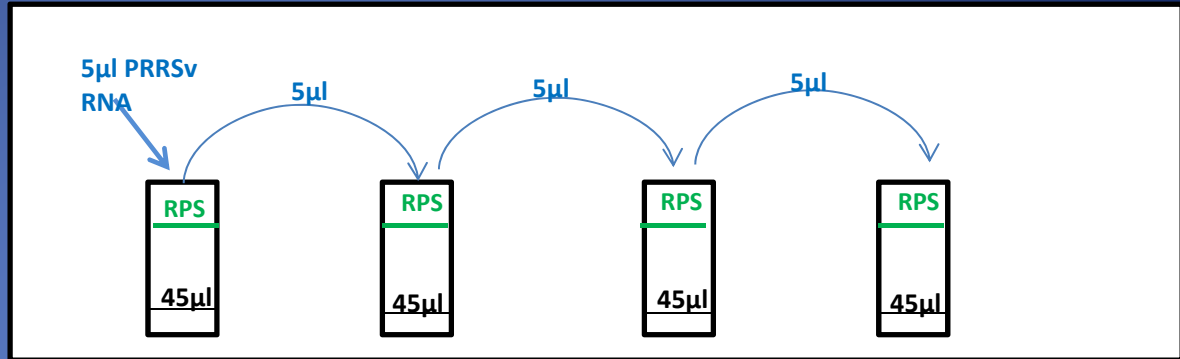
PRRSv RNA serial dilution in nuclease free water



Quantitect Probe RT-PCR kit
(Qiagen)

✓ **RT-PCR Assay
Functional**

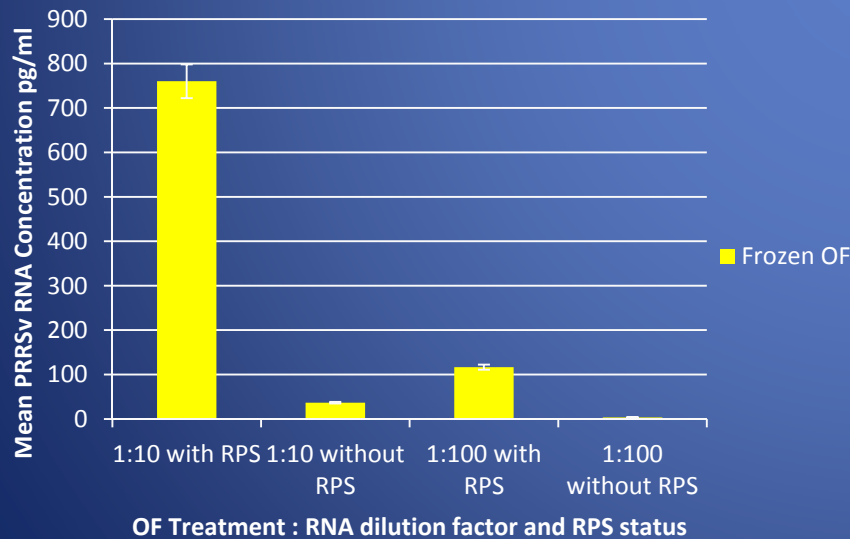
Experiment 2: PRRSv RNA spike in porcine OF with RNAProtect Saliva Reagent (RPS)



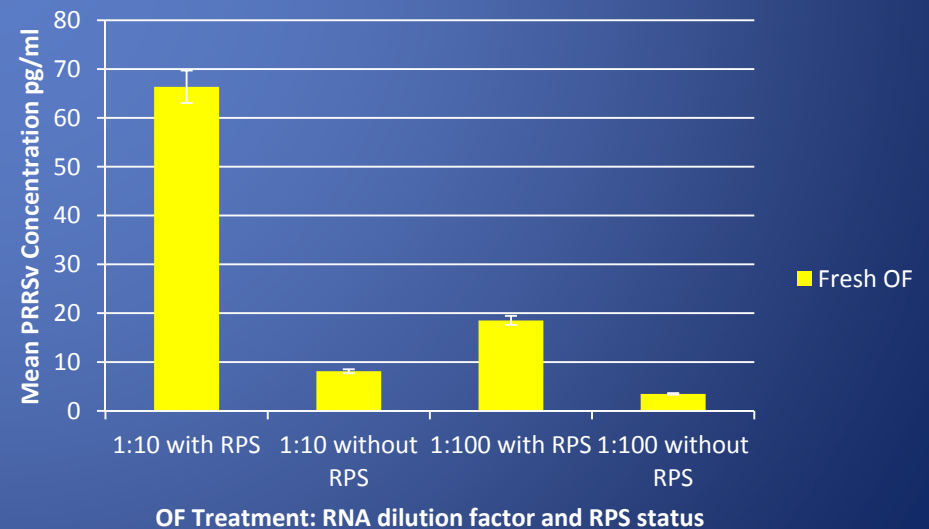
RPS Reagent (Qiagen)

RNA Extraction: RNeasy Micro kit (Qiagen)

Quantitect Probe RT-PCR kit (Qiagen)



Error bars represent 5% of the value



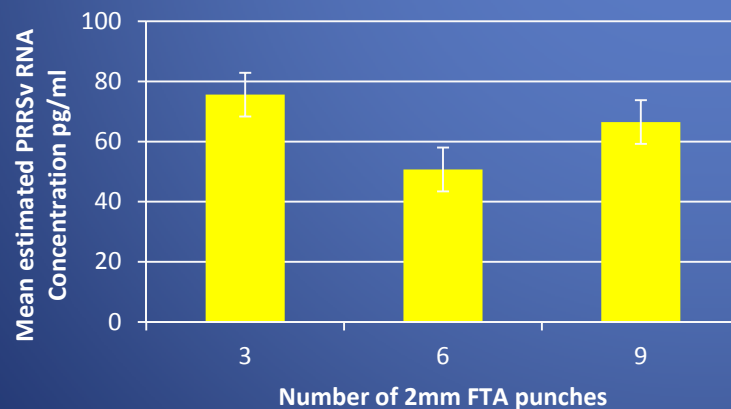
RNA concentrations were 8.2x ↑ at 1:10 & 5.3x ↑ at 1:100 dilutions, respectively

Experiment 3: PRRSv RNA spike in porcine OF on FTA Cards

Flinders Technology Associates cards (Whatman)
Storage/shipping at ambient temperatures
& recovery of nucleic acids



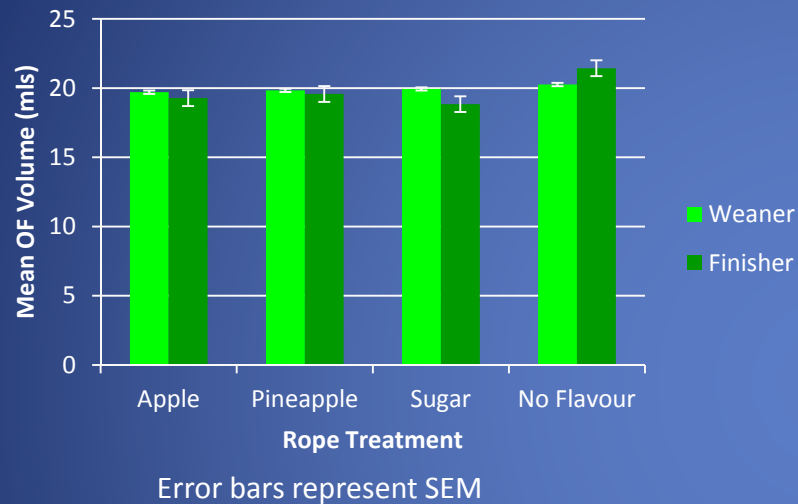
PRRSv RNA spike in fresh OF at 1:20 dilution
Inoculate onto FTA card, dry & take 2mm punches
30 min in RNA Rapid Extraction solution (Life Technologies)
QIAamp Viral RNA Mini kit (Qiagen)



Error bars represent SEM

Indication that viral RNA in porcine OF can bind to FTA cards & be recovered for RT-PCR

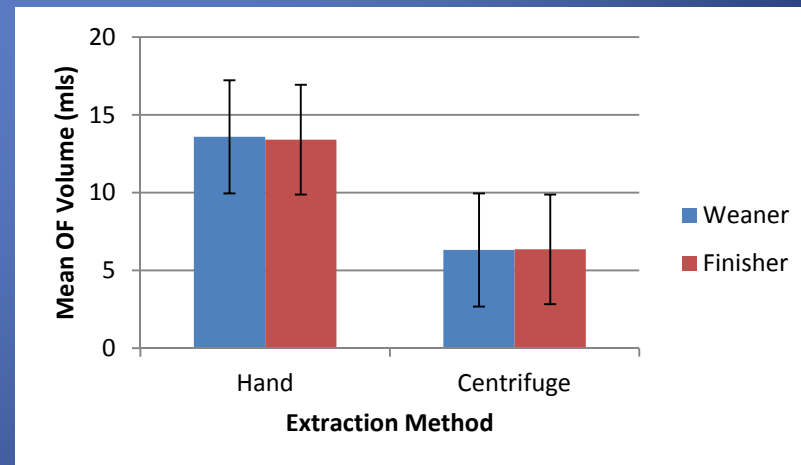
Short study: Rope additives



What's your flavour ?



- ✓ Flavours don't seem to affect salivary stimulation
- ✓ Rope training
- ✓ Sufficient OF volumes for diagnostics can be reliably extracted by hand



Implications for Industry

- ✓ RNA may be preserved at ambient temperatures in porcine OF
- ✓ Reduced time/labour costs to farmer - ↑ compliance
- ✓ Longer term benefits (FTA) → “Diagnostic archive”

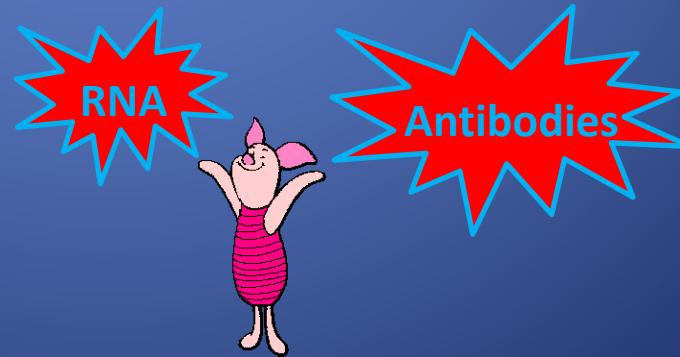


Conclusions & Next steps

- Methods used to preserve nucleic acids in human biological samples seem to work for porcine OF
- Sample treatment post collection e.g. freezing / not can influence RNA recovery

Next.....

- Extent to which RNA can be preserved in porcine OF at ambient temperatures
- Wet vs. dry methods → dry holds most potential for pig industry
- Antibodies ??



Acknowledgements

- Professor Sandra Edwards & Dr. Georg Lietz
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- The British Pig Executive



Thanks for listening !



Any Questions ?